STAT

THE ACTION OF ELECTROMAGNETIC FIELDS ON REGULATION OF *Paramecium* MOTOR FUNCTIONS

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[Text] Research on the sensitivity of *Paramecium* motor functions to the action of electromagnetic fields (EMF) is one aspect of research on EMF action at the cellular level, on the effect of EMF on selfregulating systems and, finally, on the participation of such fields in the very processes of vital cellular regulation.

A great deal of experimental information has been accumulated to date on the action of EMF on protozoan motor functions. The subjects of such research have been both orientation effects arising in response to EMF (3,5, 18,19) and the action of these fields on the body's controlling systems (9,10,12-14).

Naturally the question arises as to whether there is similarity between reactions of *Paramecium* to EMF and changes in movements of these animals under natural conditions. In other words, to what degree are EMF adequate stimuli for *Paramecium* locomotor function?

A sequence (in order of increasing voltage) of over 10 different types of movements have been noted in *Paramecium* in response to prolonged stimulation by alternating current from 50 to 50,000 Hz -- slight rotation with short, straight movements, oscillation relative to the center of the animal's long body axis, and so on (13,25). These same types of movements have been described for *Paramecium* under natural conditions (24). In this case cessation of movement in Infusoria, which is similar to electroshock reaction (ESR) described by A. S. Presman (9), is caused by reversal of cilia on half of its body (5).

In addition a bioelectric difference in potentials has been discovered in the cilia of Infusoria between the internal and external surfaces of the body. This difference changes rhythmically, synchronously with movement

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of the cilia (21). A comparison of these data permits us to consider that the EMF we employed is to some extent an adequate natural bioelectric stimulus of *Paramecium* locomotive functions.

What are the mechanisms underlying EMF action on excitable structures of *Paramecium*?

According to the enzymochemical theory, a reaction of acetylcholine with a receptor protein, which increases the permeability and serves as the starting mechanism of rapid movement of sodium and calcium ions, is the initiator of the excitation process (7). The receptor-acetylcholine complex is in dynamic equilibrium with free acetylcholine and receptor protein. The free ester is attacked by acetylcholinesterase and is quickly inactivated as a result of hydrolysis, after which the receptor (and the permeability) return to the initial rest state.

Let us examine the possible action of microwaves on the acetylcholine-cholinesterase system. The presence of active cholinesterase in the pellicle of ciliated Infusoria (27) and experimental data on participation of the acetylcholine-cholinesterase system in stimulation of *Paramecium* (6) indicate that such an approach is valid.

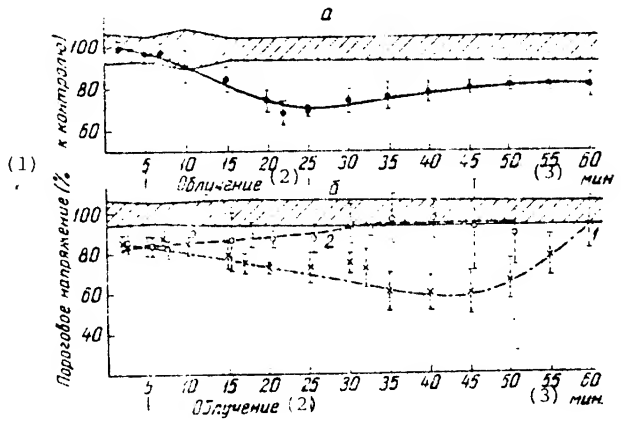
The procedure for studying *Paramecium* excitability has been described in detail in previous publications (9,13). Series of alternating current pulses with a pulse repetition frequency of 600 Hz and a series duration of 50 μsec (produced by GS-100I generator followed by electronic modulation) were used as the stimulating pulses.

Irradiation by microwaves was conducted in a special chamber, the layout of which had been described earlier in detail (14). A VNIIM i O pulse generator was used as the microwave source. Microwave irradiation was conducted in the pulse-pulse mode, the wavelength was 10 cm, the pulse repetition frequency was 700 pulses/sec, the duration of internal pulses was 1 μsec, the pulse series duration was 50 msec, and the irradiation power was 10 percent lower than that necessary for direct stimulation. The reagent solutions were prepared out of the medium in which *Paramecium* was cultured. An MBS-2 microscope was used to observe the behavior of *Paramecium*. An aluminum hood lined within by absorbent KhV-10 plates was used to protect the experimentor's eyes from microwave side effects. There was an opening in the hood for the microscope objective.

Irradiation of *Paramecium* by microwaves in this mode reduced the excitation threshold by 55 percent as compared to controls. The maximum effect was manifested 5-10 minutes after the start of irradiation (Figure 2 [sic]).

We hypothesized that by blocking cholinesterase we could disturb the dynamic equilibrium between free acetylcholine and acetylcholine bound to the receptor. In this case we suggested that the disturbance would depend on the concentration of inhibitory substance, and that it would be manifested either in an increase in the excitation threshold or in its decrease. Such a dependence on inhibitor concentration stems from the fact that when the concentration

of acetylcholine is increased, the cholinesterase activity also grows in parallel to a particular level. After this, activity begins to increase, and a high acetylcholine concentration severely inhibits enzyme action (17).



(1)

Figure 1. Change in ESR Thresholds of *Paramecium* Upon Stimulation by Alternating Current Pulses: *a* -- upon one-time microwave irradiation in continuous mode; hatched area -- variation in the threshold voltage of alternating current pulses (frequency 12 kHz, duration 50 msec) without microwave irradiation; *b* -- on the background of the action of urea in a 1-percent concentration, and upon combined action of 1-percent urea and microwave irradiation; *1* -- effect of the action of 1-percent urea; *2* -- effect of the combined action of 1-percent urea and microwave irradiation in the continuous mode.

Key:
1. Threshold voltage (percent of control)
2. Irradiation
3. Minutes

We used proserine -- a reversible inhibitor of cholinesterase activity at a concentration of $1.5 \cdot 10^{-3}$ percent as the substance blocking the action of cholinesterase. At this concentration it causes reduction of *Paramecium* excitability. The ESR threshold increases in this case by 60 percent as compared to controls (see Figure 2).

When the action of proserine (at a concentration of $1.5 \cdot 10^{-3}$ percent) is combined with microwave action, in a sense these effects are summated, as a result of which the excitation threshold is restored to its initial level during 15 minutes of irradiation (see Figure 2).

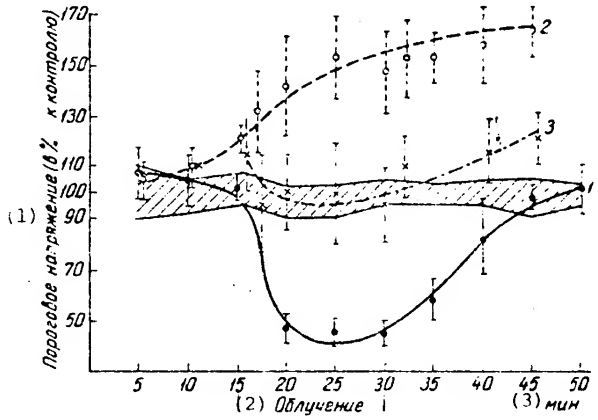


Figure 2. Change in ESR Thresholds of *Paramecium* Upon Stimulation by Alternating Current Pulses and Simultaneous Microwave Irradiation, Proserine and the Combined Action of Microwave and Proserine: *1* -- effect of irradiation by series of microwave pulses at a frequency of 700 pulses/sec and a duration of 50 msec; *2* -- effect of proserine at a concentration of $1.5 \cdot 10^{-3}$ percent; *3* -- effect of the combined action of proserine ($1.5 \cdot 10^{-3}$percent) and microwave irradiation in the pulse-pulse mode. Hatched area -- variation of the threshold voltage of alternating current pulses (frequency 600 Hz, duration 50 msec) without microwave irradiation. [Key: See key, Figure 1]

Thus microwave irradiation acting on a background of reduced *Paramecium* excitability in a sense cancels out the effect of excitability reduction, normalizing the ESR thresholds, probably due to restoration of the disturbed dynamic equilibrium between the acetylcholine-receptor complex and free acetylcholine, or between free acetylcholine and cholinesterase. To verify the action that microwave irradiation would have on the second component of the starting reaction -- the protein, we made use of the possibilities for actively changing the excitability level of *Paramecium* using SH-group donors and acceptors (4,8,15).

The research procedures were similar to those described above with the single difference that microwave irradiation was conducted in continuous mode, using a wavelength of 16 cm (produced by a GCh-8 generator), specific power of 5 mw/cm^3, and an irradiation time of 20 minutes.

Microwaves irradiation in this mode reduces the excitation threshold by 30 percent, the maximum effect being manifested in the last 5 minutes of irradiation during the aftereffect period (Figure 1).

A thiolic toxin -- mercuric chloride -- at a concentration of $1.5 \cdot 10^{-5}$ was used to bind the SH-groups of the proteins. In response to this reagent the *Paramecium* excitation threshold doubled the control threshold in 45 minutes.

When *Paramecium* is subjected to simultaneous action of microwave radiation and mercuric chloride in the concentration indicated above, a 10-15 percent reduction is observed in the excitation threshold as compared to the excitation threshold at the same moments in time in the presence of mercuric chloride alone. In this case this effect persists for 20 minutes after irradiation (Figure 3*a*). Thus microwave irradiation partially cancels out the effect of mercuric chloride -- that is, in this case it normalizes the excitation threshold to a certain degree.

Inasmuch as thiolic toxins can, in addition to binding with SH-groups, have an inhibitory action on various enzyme systems, we used an oxidation reaction to inhibit SH-groups. For this purpose we employed oxidized cysteine, which is one of the metabolites formed during normal vital activity.

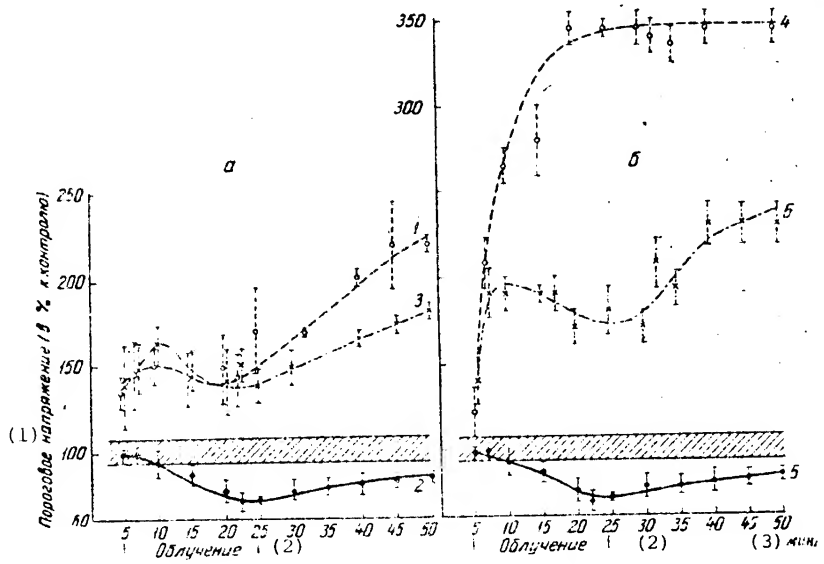


Figure 3. Change in ESR Threshold of *Paramecium* Upon Stimulation by Alternating Current Pulses and Simultaneous Microwave Irradiation: *а* -- in the presence of mercuric chloride and combined action of microwaves and mercuric chloride: *1* -- effect of mercuric chloride at a concentration of $1.5 \cdot 10^{-5}$ percent; *2* -- effect of irradiation by continuous microwaves; *3* -- effect of the combined action of mercuric chloride ($1.5 \cdot 10^{-5}$ percent) and

microwave irradiation in continous mode; hatched area -- variation in threshold voltage of alternating current pulses (frequency 12 kHz, duration 50 msec) without microwave irradiation. *b* -- in the presence of cysteine and combined action of cysteine and microwave irradiation; *4* -- effect of cysteine in a 1-percent concentration; *5* -- effect of irradiation by continuous microwaves; *6* -- effect of the combined action of cysteine (1 percent) and microwave irradiation in the continuous mode; hatched area -- variation in threshold voltage of alternating current pulses (frequency 12 kHz, duration 50 msec) without microwave irradiation.

Key:
[See Key, Figure 1]

When cysteine at concentrations of $1\text{-}1.5 \cdot 10^{-5}$ percent is added to the *Paramecium* medium, an excess of mercaptide RS^- ions, which oxidize more readily than do undissociated SH-groups, arises within it. Since the oxidation optimum of cysteine lies in the alkaline pH (16) and the *Paramecium* medium has a weakly alkaline reaction (pH 7.5-8.1), and the concentration of mercaptide ions is rather large in the medium, cysteine oxidation would occur in the following way:

$$RS^- \rightarrow RS. + e^- \qquad \text{I}$$
$$RS. + RS. \rightarrow RS\text{-}RS; \qquad \text{II}$$

In this case the rate of the oxidation process under these conditions would be defined by reaction II. The disulfide compounds that are formed may in turn cause oxidation of the SH-groups of *Paramecium* proteins -- that is, they may promote configurational changes in the proteins.

In the indicated concentrations, cysteine evokes a rise in the *Paramecium* excitation thresholds, the degree to which this effect is pronounced depending on the cysteine concentration: When cysteine is at a concentration of $1.5 \cdot 10^{-5}$ percent the ESR threshold increases by 45 percent, while when the concentration is 1 percent the threshold increases by 250 percent (Figure 3*b*).

When *Paramecium* is subjected to microwave irradiation on a background of cysteine action at concentrations from $1.5 \cdot 10^{-3}$ to $1.5 \cdot 10^{-5}$ percent, the excitation threshold becomes fully restored, rising in responce to cysteine. When higher concentrations of cysteine are employed ($1\text{-}1.5 \cdot 10^{-4}$ percent) in combination with microwave irradiation the excitation threshold drops by a factor of two during irradiation as compared to the size of the excitatior threshold at the same moments in time in the presence of cysteine alone (Figure 3*b*).

Thus depending on cysteine concentration, microwave irradiation partially or completely cancels out the effect of cysteine -- that is, it normalizes excitability, apparently hindering oxidation of protein SH-groups.

Data on increasing resistance of animals to the action of ionizing radiation as a result of preliminary microwave irradiation (11,12) can serve as indirect evidence for such a hypothesis. The effect stems from the evoked action of microwaves, reduction of the oxygen partial pressure in arterial blood, and inhibition of electron excitation in protein molecules. As a consequence of these processes the formation of free radicals in response to ionizing radiation is prevented or reduced (22). We can suggest on the basis of these experiments that microwaves affect SH-groups. To test these hypotheses we attempted to increase the number of reactive SH-groups in protein of *Paramecium* itself.

We used urea as the agent freeing SH-groups of native proteins. As in the case of just microwave action alone (Figure 1*a*), when a 4-percent solution of urea is introduced into the *Paramecium* medium the excitation threshold of the Infusoria drops. Urea action is manifested in the very first minutes, reaching a maximum at 35 minutes (the ESR threshold drops by 40 percent). At 60 minutes the excitation threshold reaches its initial level (Figure 1*b*).

When *Paramecium* is subjected to simultaneous action of urea and microwave irradiation the excitation threshold, which is altered by urea, is restored to its initial level after 20 minutes of irradiation. Although as had been described above (Figure 1*a*), microwave irradiation in this mode can itself raise *Paramecium* excitability, when these two factors are combined their unidirectional effects are totally canceled out.

Thus normalization of the excitability level is a general effect of microwaves, irrespective of whether the particular agent reduces (proserine, mercuric chloride, cysteine) or raises (urea) *Paramecium* excitability.

Although the regulating effect of electromagnetic fields is obvious in this case, we can discuss its mechanism only hypothetically.

Biochemical research has shown that protein molecules in protozoan cilia and flagella exist simultaneously in α and β-configurations (2), and that any destructive changes (for example, the action of urea) disturb the initial ratio between these configurations.

It would be natural to hypothesize that by canceling the effect of urea, microwave irradiation can restore this equilibrium. Data on change in the ratio of the two possible states or configurations of macromolecular systems in response to high-energy electric fields (20) argue in favor of this hypothesis. An electric field shifts the equilibrium between the two states in the direction of greatest polarization and changes the elasticity of protein chains. The latter, in particular, may be at the basis of muscular contraction. Consequently it is fully probable that β-configuration proteins transform into the stabler α-configuration in response to EMF.

At the same time when protein solutions are irradiated by microwaves all polarized side chains of such molecules would become oriented along the field lines, exhibiting a tendency for breakage of hydrogen bonds and changing the hydration zone (26). This can apparently only partially explain the external analogy between the effects of microwaves and urea, but not their joint action.

Thus we can say on the basis of the obtained data that in all probability electromagnetic fields act on both components examined by us of the acetylcholine-receptor protein starting reaction.

Conclusions

1. Electromagnetic fields, including microwaves, are active and apparently adequate stimuli for *Paramecium*.

2. The action of electromagnetic fields is based on the association between locomotor functions and electromagnetic regulation.

3. Obviously the action of EMF in the microwave range is manifested at the level of enzyme processes in the pellicle of *Paramecium* (the acetylcholine-cholinesterase system), and in the form of configurational changes in proteins of the acetylcholine-receptor protein complex.

In conclusion I express my gratefulness to Candidate of Biological Sciences A. S. Presman for his constant attention and assistance in completion of this project.

BIBLIOGRAPHY

1. Dogel', B. A., "Obshchaya Protistologiya" (General Protistology), Moscow, Izd-vo Sovetskaya Nauka, 1951.

2. Inue, Sh., "Mobility of Cilia and Flagella, and the Mitosis Mechanism," in "Sovremennyye Problemy Biofiziki" (Modern Problems of Biophysics), Vol 2, Moscow, IL.

3. Kogan, A. B., and Tikhonova, N. A., "Action of a Constant Magnetic Field on *Paramecium* Movement," BIOFIZIKA, Vol 10, No 2, 1965, p 292.

4. Koshtoyants, Kh. S., "Belkovyye Tela, Obmen Veshchestv, I Nervnaya Regulyatsiya" (Protein Bodies, Metabolism, and Nervous Regulation), Moscow, Izd-vo AN SSSR, 1951.

5. Koshtoyants, Kh. S., "Osnovy Sravintel'noy Fiziologii" (Fundamentals of Comparative Physiology), Vol 2, Moscow, Izd-vo AN SSSR, 1957.

6. Koshtoyants, Kh. S., and Kokina, N. N., "The Role of the Acetylcholine-Cholinesterase System in Galvanotaxis Phenomena and Summation of Stimuli in *Paramecium*," BIOFIZIKA, Vol 2, No 1, 1957, p 46.

7. Nakhmanzon, D., "The Future of Research on Molecular Principles of Nerve Activity," in "Molekulyarnaya Biologiya" (Molecular Biology), Moscow, Izd-vo Nauka, 1964.

8. Popova, M. F., "Effect of SH-Group Blocking on *Paramecium* Galvanotaxis," TR. IN-TA MORFOL. ZHIVOTNYKH AN SSSR, Vol 6, 1952.

9. Presman, A. S., "Excitability of *Paramecium* in Response to Stimulation by Constant and Alternating Current Pulses," BIOFIZIKA, Vol 8, No 1, 1963, p 138.

10. Presman, A. S., "Action of Microwaves on *Paramecium*," BIOFIZIKA, Vol 8, No 2, 1963, p 258.

11. Presman, A. S., and Levitina, N. A., "Effect of Nonthermal Microwave Irradiation on Resistance of Animals to Gama-Radiation," RADIOBIOLOGIYA, Vol 2, No 1, 1962, pp 170-171.

12. Presman, A. S., and Rappeport, S. M., "New Data on the Existence of an Excitatory System in *Paramecium caudatum*. 1. Reaction of *P. caudatum* to Direct Current Pulses," NAUCHN. DOKL. VYSSH. SHKOLY, BIOL. NAUKI, Vol 1, 1964, p 52.

13. Presman, A. S., and Rappeport, S. M., "New Data on Existence of an Excitatory System in *Paramecium caudatum*. 2. Reaction of *P. Caudatum* to Alternating Current Pulses," NAUCHN. DOKL. VYSSH. SHKOLY, BIOL. NAUKI, Vol 3, 1964, p 44.

14. Presman, A. S., and Rappeport, S. M., "Action of Microwaves on the Excitatory System of *Paramecium*," BYULL. EKSPERIM. BIOL. I MED., Vol 4, 1965, p 48.

15. Smirnova, N. A., "The Dependence of Galvanotaxis of *Paramecium* on Sulfhydryl Groups," BIOFIZIKA, Vol 2, No 6, 1957, p 670

16. Torchinskiy, Yu. M., "Properties of SH- and SS-Groups and the Causes of Altered Reactivity of These Groups in Proteins," USP. SOVREM. BIOL., Vol 55, No 2, 1963, pp 161-179.

17. Shtraub, F., "Biokhimiya" (Biochemistry), Budapest, 1963.

18. Brown, F. A., "Responses of the Planarian, Dugezia, and the Protozoan, *Paramecium* , to Very Weak Horizontal Magnetic Fields," BIOL. BULL., Vol 23, No 2, 1962, p 264-281.

19. Heller, J., and Teixevia-Pinto. A., "A New Physical Method of Creating Chromosomal Aberation," NATURE, Vol 183, 1959, p 905.

20. Hill, T., "Some Possible Biological Effects of Action of the Electrical Field on Nucleic Acids and Proteins," J. AMER. CHEM. SOC., Vol 80, 1958, pp 2142-2147.

21. Kinosita, H., "Electric Potentials and Ciliary Response in Opalina," J. FAC. SCI., UNIV. TOKYO, Vol 7, 1954, p 1.

22. Michaelson, S. M., Thomson, R. A. E., and Howland, J. W., "The Influence of Microwaves on Ionizing Radiation Exposure," AEROSPACE MED., Vol 34, No 2, 1963, pp 111-115.

23. Parducz, B., "Reizphysiologische Untersuchungen an Ziliaten. I. Uber das Aktions-system von *Paramecium*," ACTA MICROBIOL. ACAD. SCIENT. RUNG., Vol 1, 1954, pp 1-3.

24. Parducz, V., "Reizphysiologische Untersuchungen an Ziliaten. VII. Das Problem der Vorbestimmten Leitungsbahnen," ACTA BIOL. HUNG., Vol 8, 1958, p 219.

25. Scheminzky, Fe. u. Fr., and Buketsch, F., "Electro-Taxis, Electro-Tropismus, Electro-Narkose, und verwande Erscheinung," TABLES-BIOL., Vol 19, 1941, p 114.

26. Schwan, H., "Molecular Response Characteristics to Ultra-High Frequency Fields," "Proc. wnd Tri-Serv. Conf. Biol. Eff. Microwaves," 1958, pp 33-48.

27. Seaman, G., "Localization of Acethylcholinesterase Activity in the Protozoan *Tetrahymena geleii*," PROC. SOC. EXPTL. BIOL. AND MED., Vol 76, 1951, p 169.

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